Synthesis, Characterization and Biological Evaluation of Cationic Organoruthenium(II) Fluorene Complexes: Influence of the Nature of the Counteranion

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Table S1. Elemental analysis data for **1**, **2**, **3**[X], **4**, and **5**[BF_4].

	Content calculated (%)			Content found (%)		
	C	H	N	C	H	N
1	84.42	5.22	10.36	82.40	5.31	10.00
2	85.94	5.11	4.18	84.73	5.29	4.30
3[BF_4]	52.52	3.53	4.90	52.17	3.46	5.00
3[PF_6]	47.67	3.20	4.45	45.56	3.33	4.90
3[BPh_4]	73.18	5.01	3.48	69.29	4.95	3.30
4	65.87	3.69	2.56	62.88	4.03	2.50
5[BF_4]	61.87	4.01	4.12	59.29	4.11	4.10

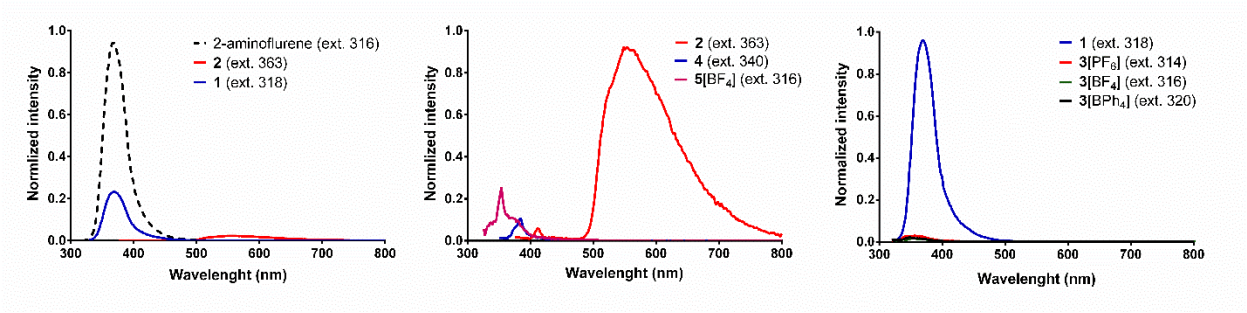


Figure S1. Fluorescent spectra of 25 μM solutions of 2-aminofluorene, ligands, and ruthenium complexes in water (0.25% DMSO) at their maximum excitation wavelength.

Table S2. Wavelengths of maximum fluorescence excitation and emission of 2-aminofluorene, ligands, and ruthenium complexes in water (0.25% DMSO).

Compound	Maximum λ_{ex} (nm)	Maximum λ_{em} (nm)
2-aminofluorene	316	364
1	318	370
2	363	556
3[BF_4]	316	352
3[PF_6]	314	350
3[BPh_4]	320	360
4	340	384
5[BF_4]	316	354

Table S3. Crystallographic data and structure refinement for complexes **3**[BF_4], **3**[BPh_4] and **4**.

	3[BF_4]	**3**[BPh_4]	**4**
empirical formula	$C_{25}H_{20}BClF_4N_2Ru$	$C_{49}H_{40}BClN_2Ru$	$C_{30}H_{22}ClNORu$
formula weight	571.76	804.16	549.00
crystal size	0.28 x 0.08 x 0.06 mm	0.13 x 0.1 x 0.04 mm	0.08 x 0.04 x 0.04 mm
crystal system, space group	orthorhombic, $P2_12_12_1$	monoclinic, *C* 1 2/*c* 1	monoclinic, *P* 2_1/n
unit cell dimensions	a = 6.4160(3)Å	a = 18.2318(7)Å	a = 9.9911(3)Å
	b = 16.3665(8)Å	b = 13.1222(5)Å	b = 11.2130(3)Å
	c = 20.9513(10)Å	c = 33.2897(14)Å	c = 20.5629(6)Å
	α = 90º	α = 90º	α = 90º
	β = 90	β = 105.192(2)º	β = 94.9430(10)º
	γ = 90º	γ = 90º	γ = 90º
volume	2200.04(18) $Å^3$	7686.0(5) $Å^3$	2295.10(11) $Å^3$
Z, Calculated density	4, 1.726 Mg m^{-3}	8, 1.390 Mg m^{-3}	4, 1.589 Mg m^{-3}
F(000)	1144.0	3312.0	1112.0
μ	7.342 mm^{-1}	2.842 mm^{-1}	4.509 mm^{-1}
temperature	100 K	150 K	100 K
wavelength	1.54176Å (CuK_α)	1.34139Å (GaK_α)	1.34139Å (GaK_α)
index ranges	$-7 \le h \le 7$	$-22 \le h \le 22$	$-12 \le h \le 12$
	$-20 \le k \le 20$	$-16 \le k \le 16$	$-14 \le k \le 14$
	$-25 \le l \le 25$	$-41 \le l \le 40$	$-26 \le l \le 26$
θ range for data collection	6.854 to 144.424°	3.656 to 57.431°	7.508 to 121.46°
reflections collected/unique	60323/4315	7900/6581	45347/5275
data/parameters/restraints	4315/387/170	6581/594/480	5275/307/0
Goodness-of-fit on F^2	1.248	1.145	1.060
Final R indices [$I > 2\sigma(I)$] [a,b]	R1 = 0.0584	R1 = 0.0751	R1 = 0.0286
	*w*R2 = 0.1382	*w*R2 = 0.1754	*w*R2 = 0.0736
R indices (all data)	R1 = 0.0585	R1 = 0.0870	R1 = 0.0325
	*w*R2 = 0.1383	*w*R2 = 0.1820	*w*R2 = 0.0768
Largest diff. peak and hole	0.69 and -0.72 e $Å^{-3}$	1.200 and -0.992 e $Å^{-3}$	1.73 and -0.25 e $Å^{-3}$
CCDC deposition no.	1888978	1888979	1888980

[a] $R_1 = \Sigma||F_o| - |F_c|| / \Sigma|F_o|$. [b] $wR_2 = \{\Sigma[w(F_o^2 - F_c^2)^2] / \Sigma[w(F_o^2)^2]\}^{1/2}$.

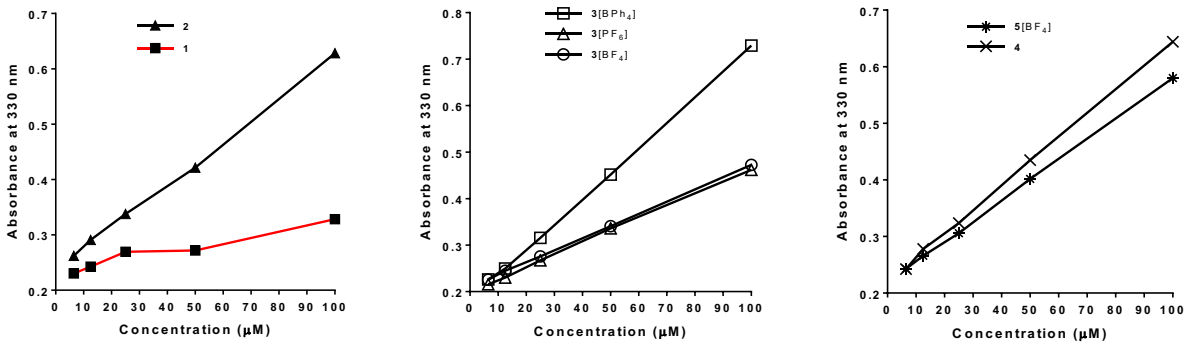


Figure S2. Absorbance intensity measurement (at 330 nm) of **1**, **2**, **3**[X], **4** and **5**[BF_4] solutions at various concentrations. Only **1** shows a non-linear increase in absorbance intensity with an increase in compound concentration.

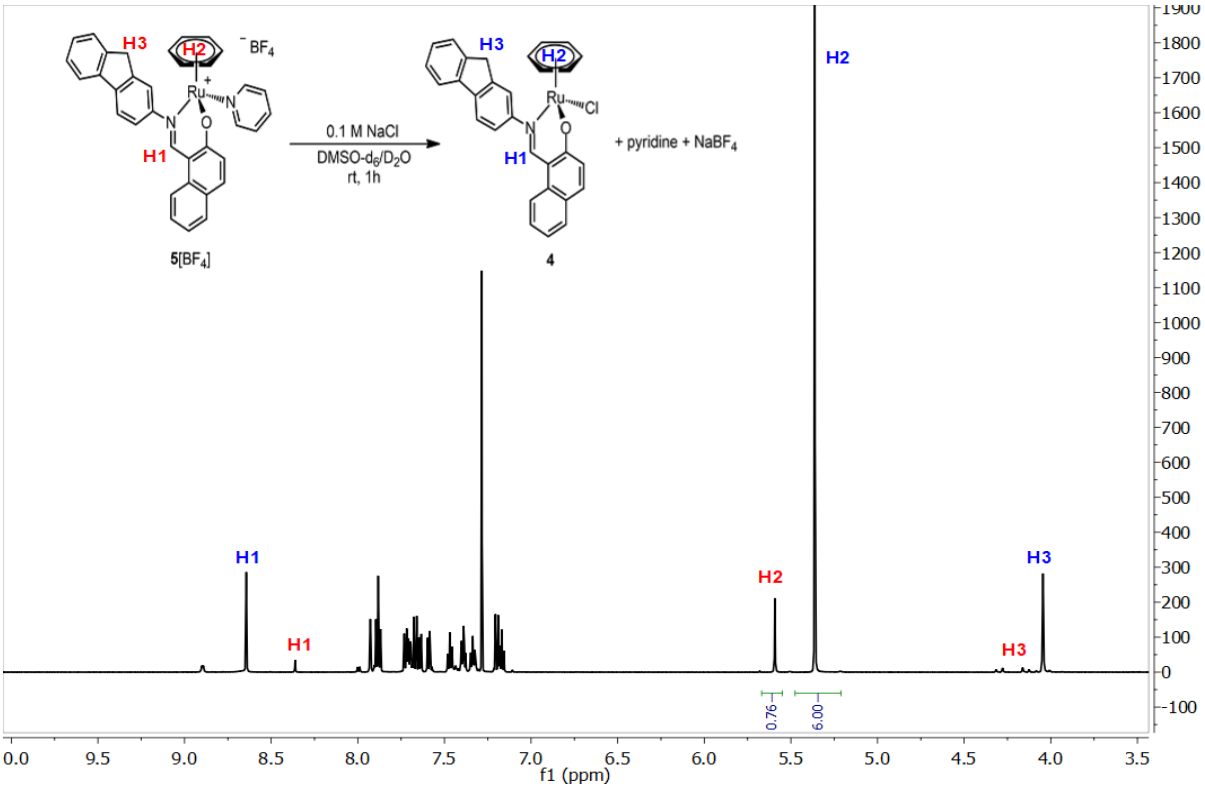


Figure S3. ^{1}H NMR ($CDCl_3$) showing conversion (~89%) of **5**[BF_4] to **4** in the presence of 0.1 M NaCl in DMSO-d_6/D_2O mixture. The details for this NMR experiment are reported in the Experimental section.

Table S4. IC_{50} values determined for 2-aminofluorene, ligands, $Na[BPh_4]$, $Na[BF_4]$, and pyridine against MCF-7 and T47D cell lines.

	IC_{50} (μM)	
	MCF-7	T47D
2-aminofluorene	>100	>100
1	84.9 (±8.0)	95.8 (±1.5)
2	22.1 (±0.2)	21.5 (±0.2)
$Na[BPh_4]$	50.1 (±1.4)	>100
$Na[BF_4]$	>100	>100
pyridine	>100	>100

[a] Inhibitory activity was determined by exposure of cell lines to 200 μL solution of each complex for 48 h and expressed as the concentration required to inhibit cell viability by 50% (IC_{50}). Errors correspond to the standard deviation of three independent experiments.

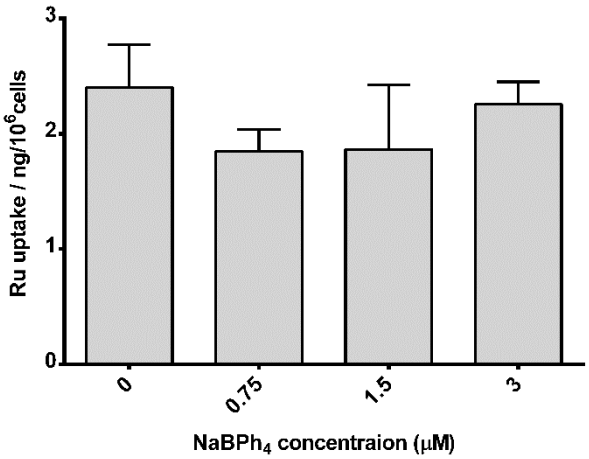
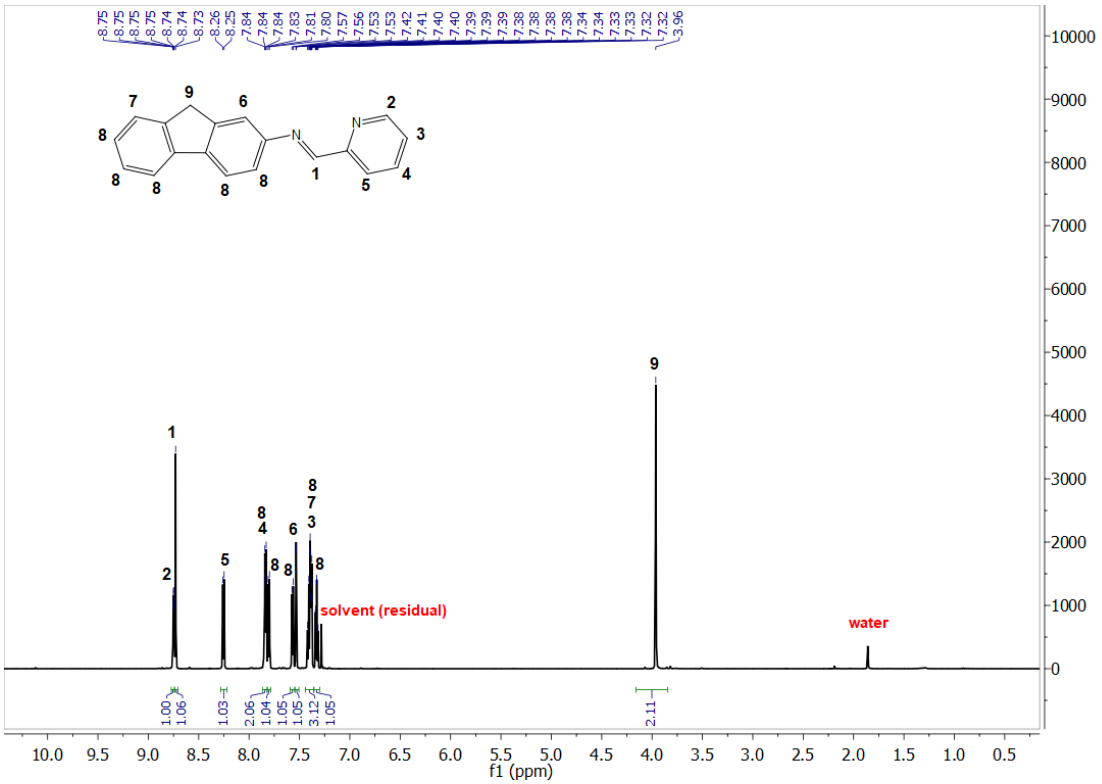


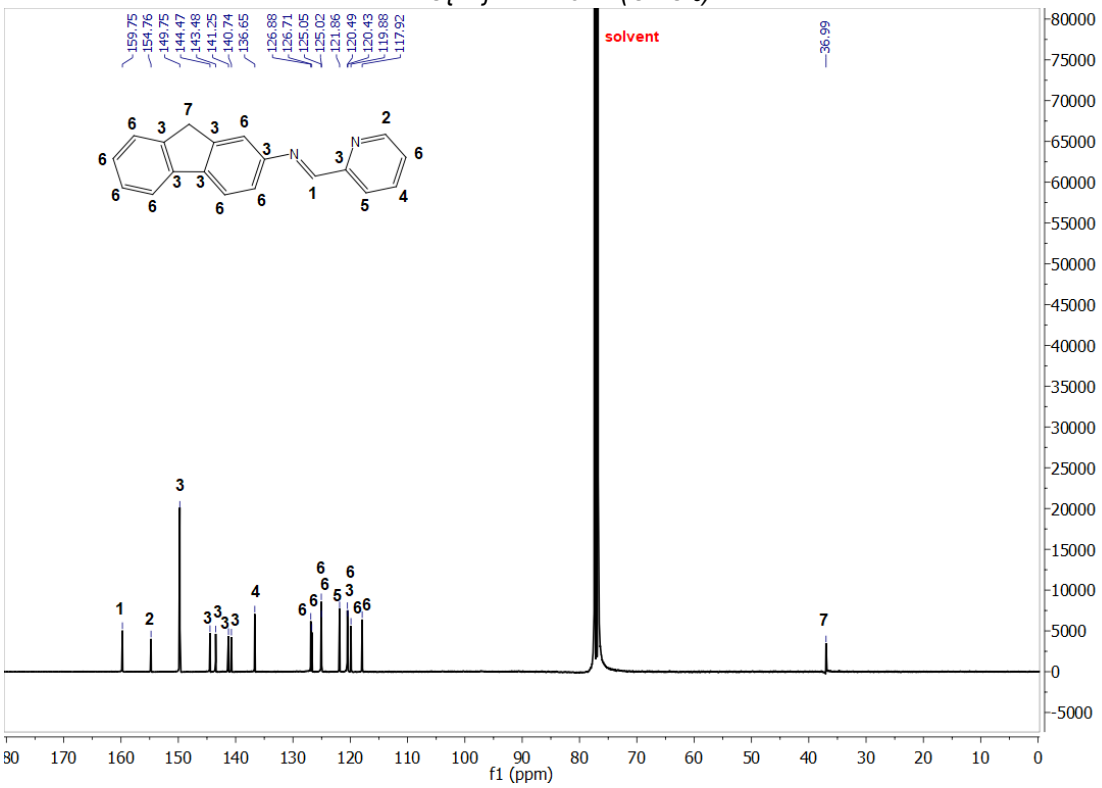
Figure S4. Ruthenium cellular uptake, determined by ICP-MS, after exposure of MCF7 cells to a **3**$[BF_4]$ 3 μM solution alone, or to a 3 μM co-treatment of **3**$[BF_4]$ and $NaBPh_4$. Error bars in the graph represent the standard deviation (n=3).

^{1}H, $^{13}C\{^{1}H\}$, ^{19}F, COSY, and HSQC NMR spectra

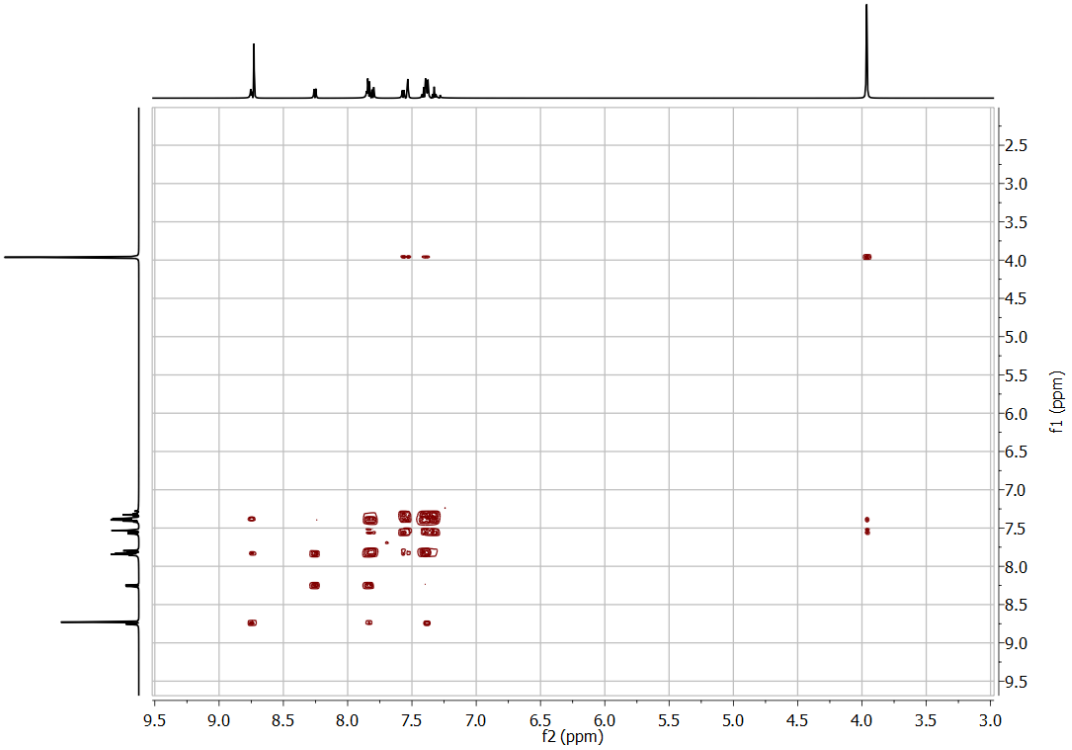
*^{1}H NMR of **1** ($CDCl_3$)*



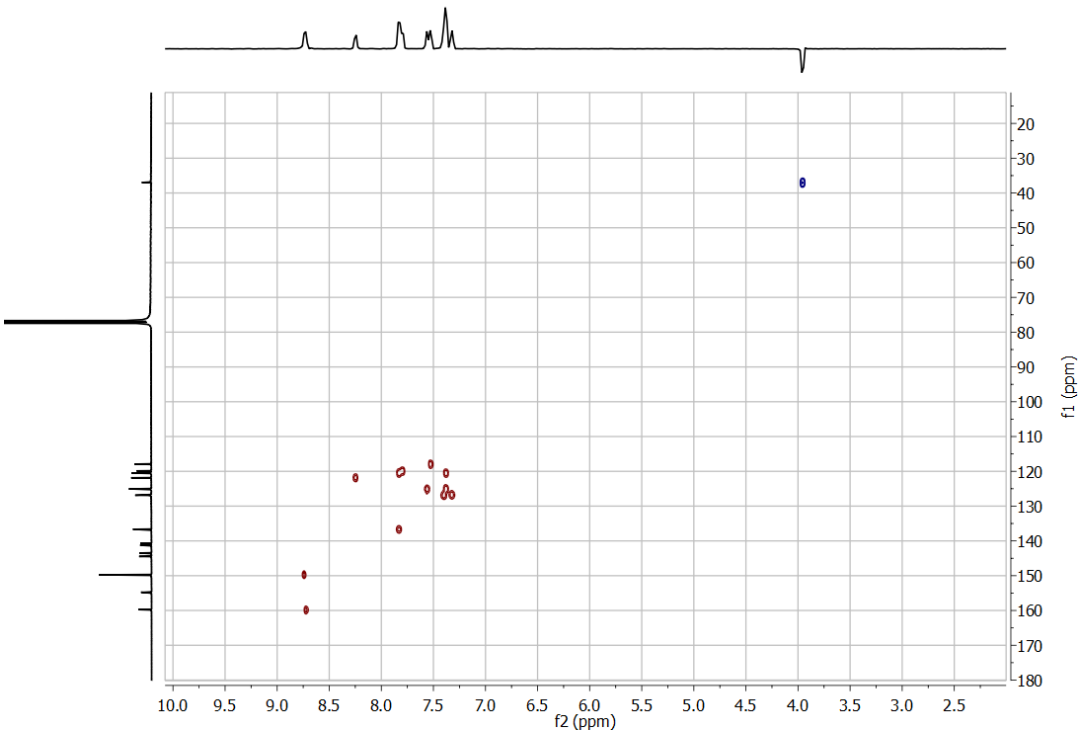
*$^{13}C\{^{1}H\}$ NMR of **1** ($CDCl_3$)*



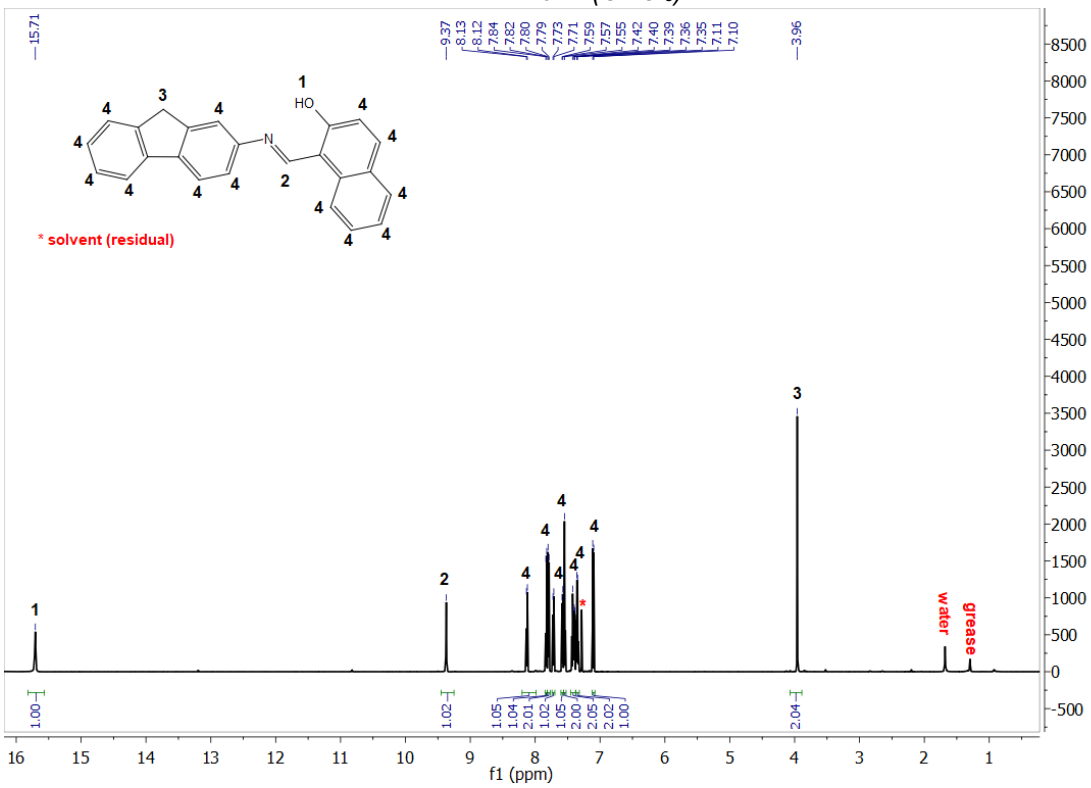
*COSY of **1** ($CDCl_3$)*



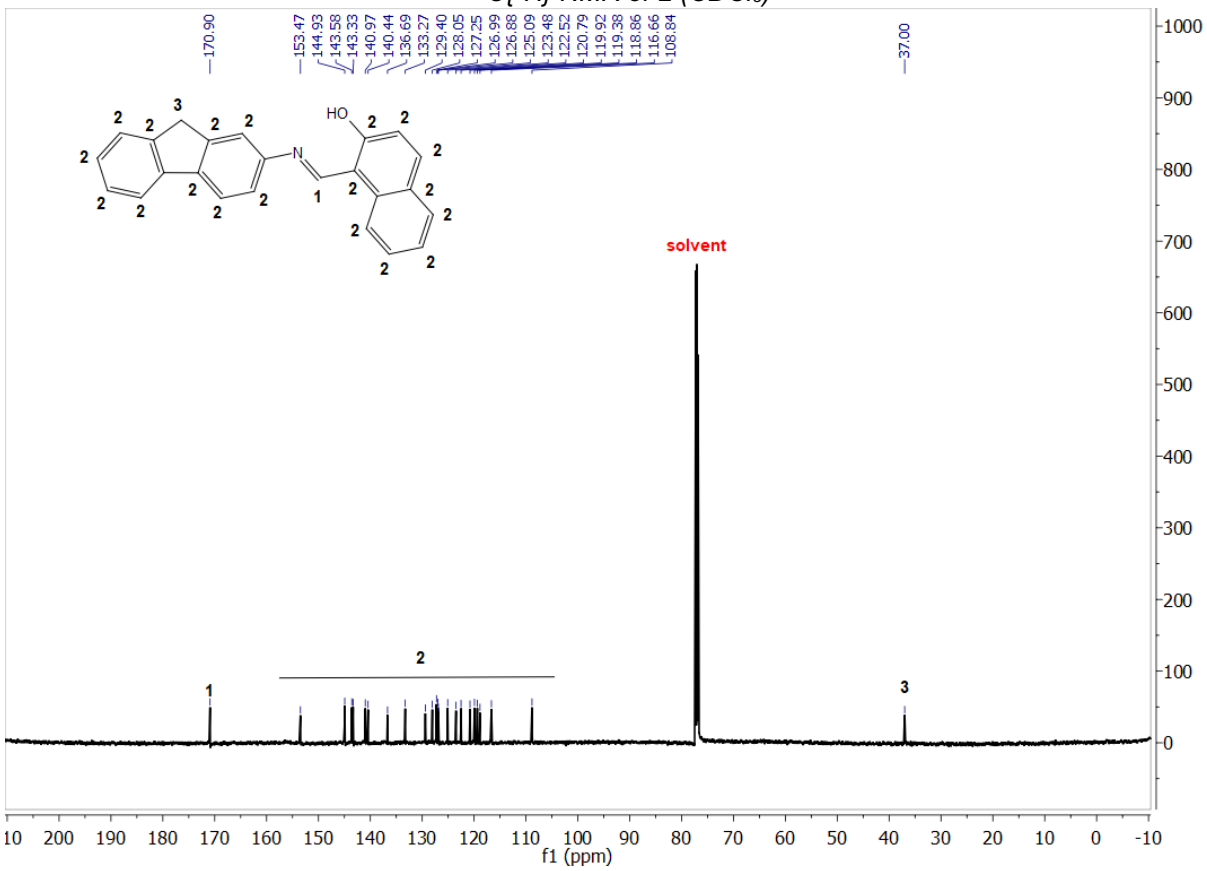
*HSQC of **1** ($CDCl_3$)*



*^{1}H NMR of **2** ($CDCl_3$)*



*$^{13}C\{^{1}H\}$ NMR of **2** ($CDCl_3$)*



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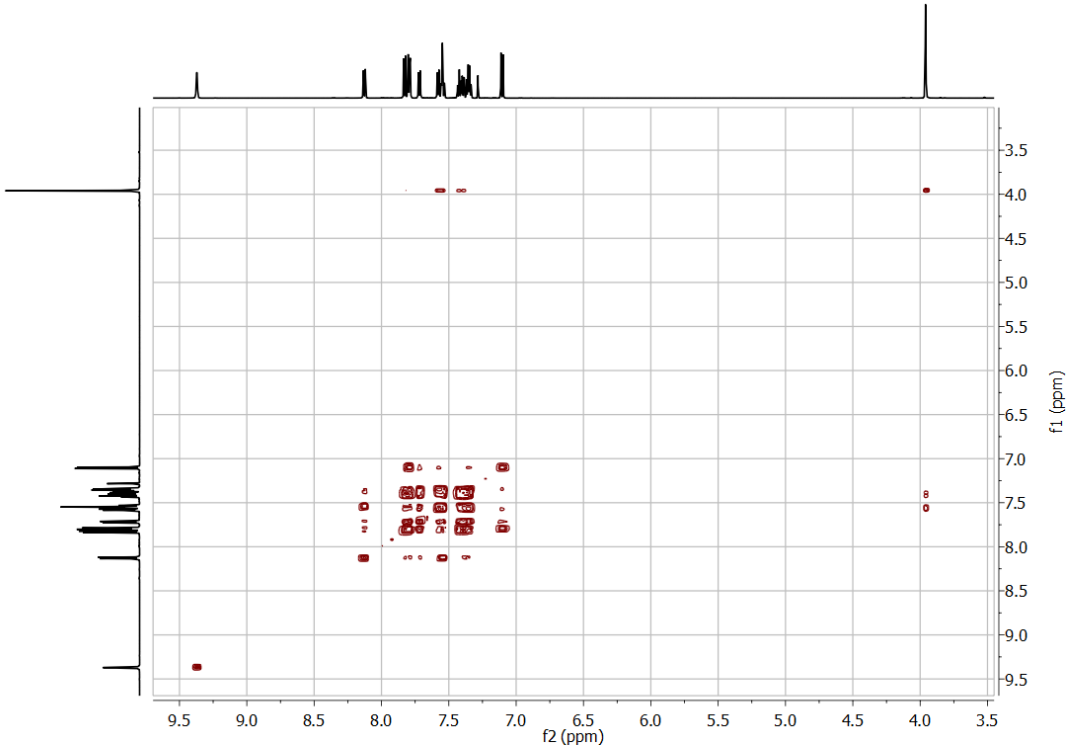
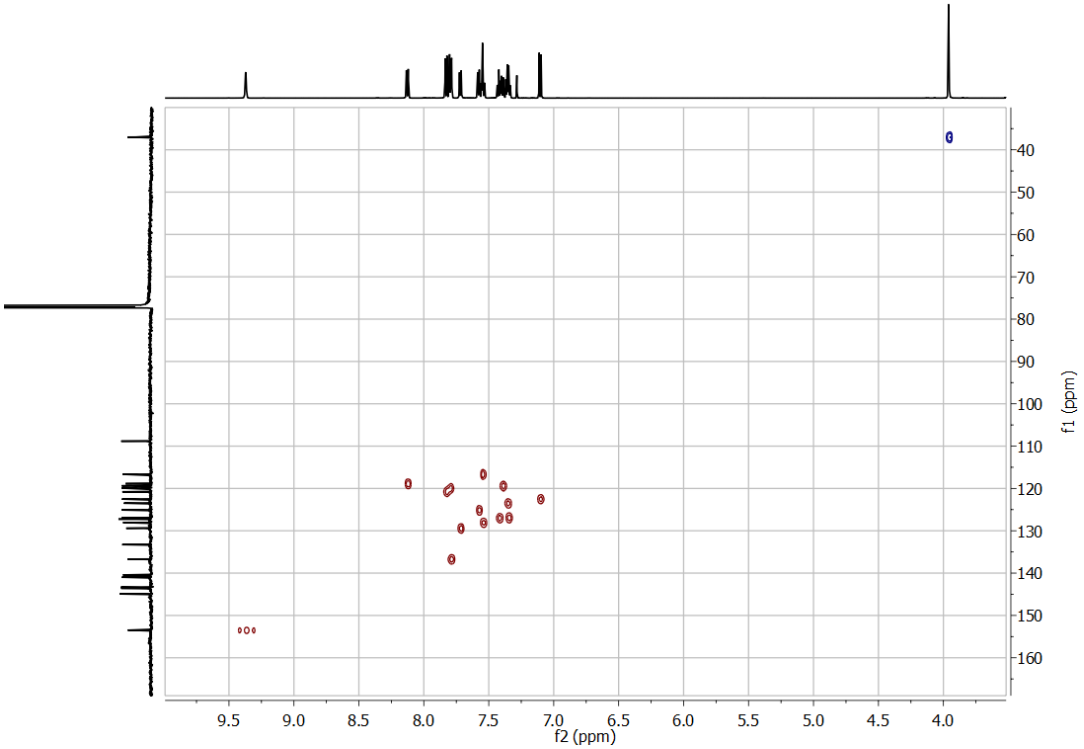
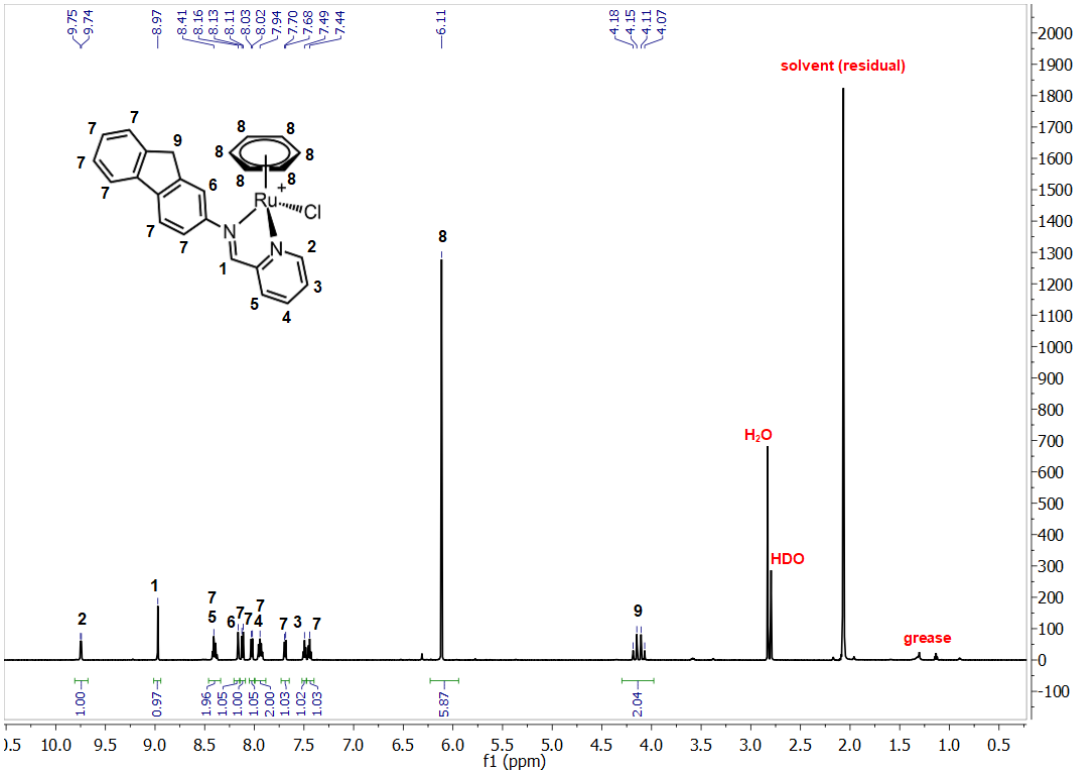


*HSQC of **2** ($CDCl_3$)*



*^{1}H NMR of **3**[BF_4] and **3**[PF_6] (acetone-d_6)*



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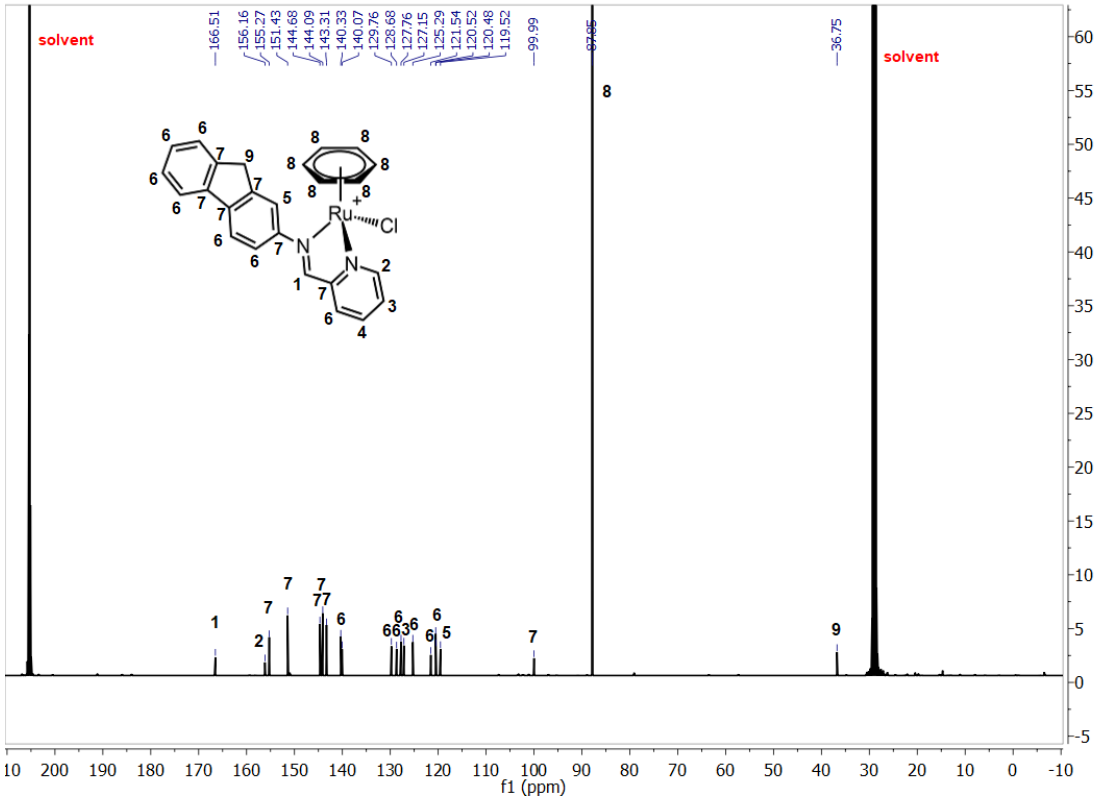

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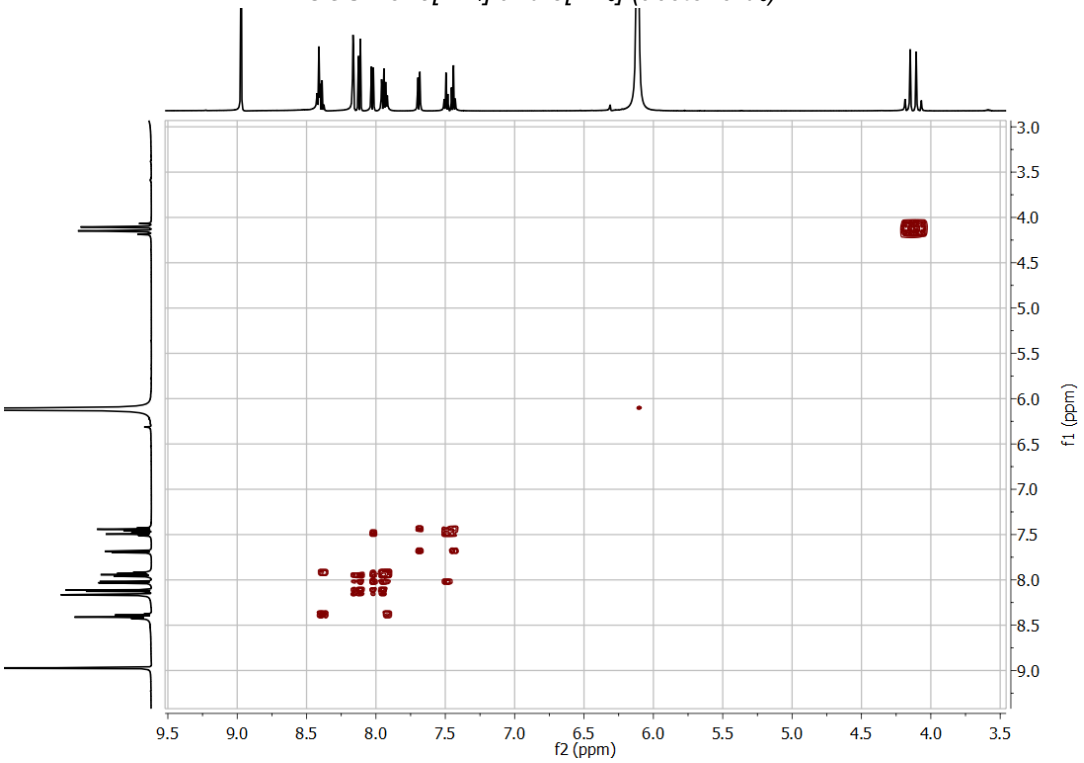
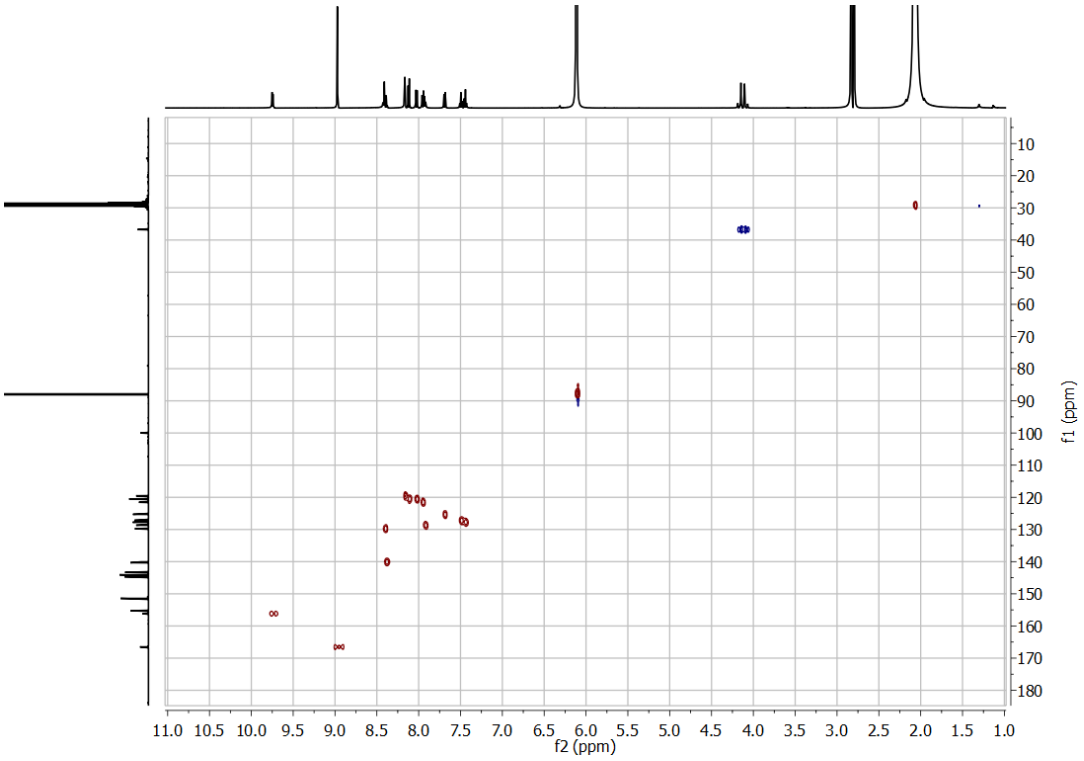
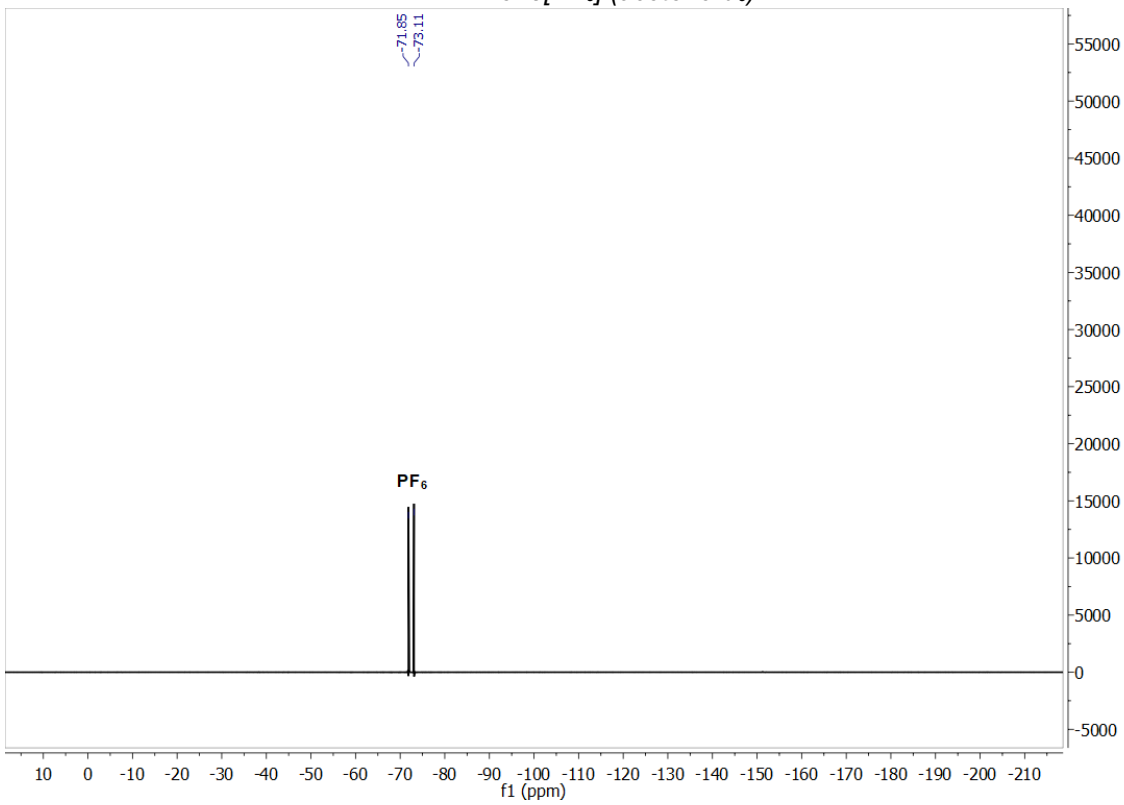


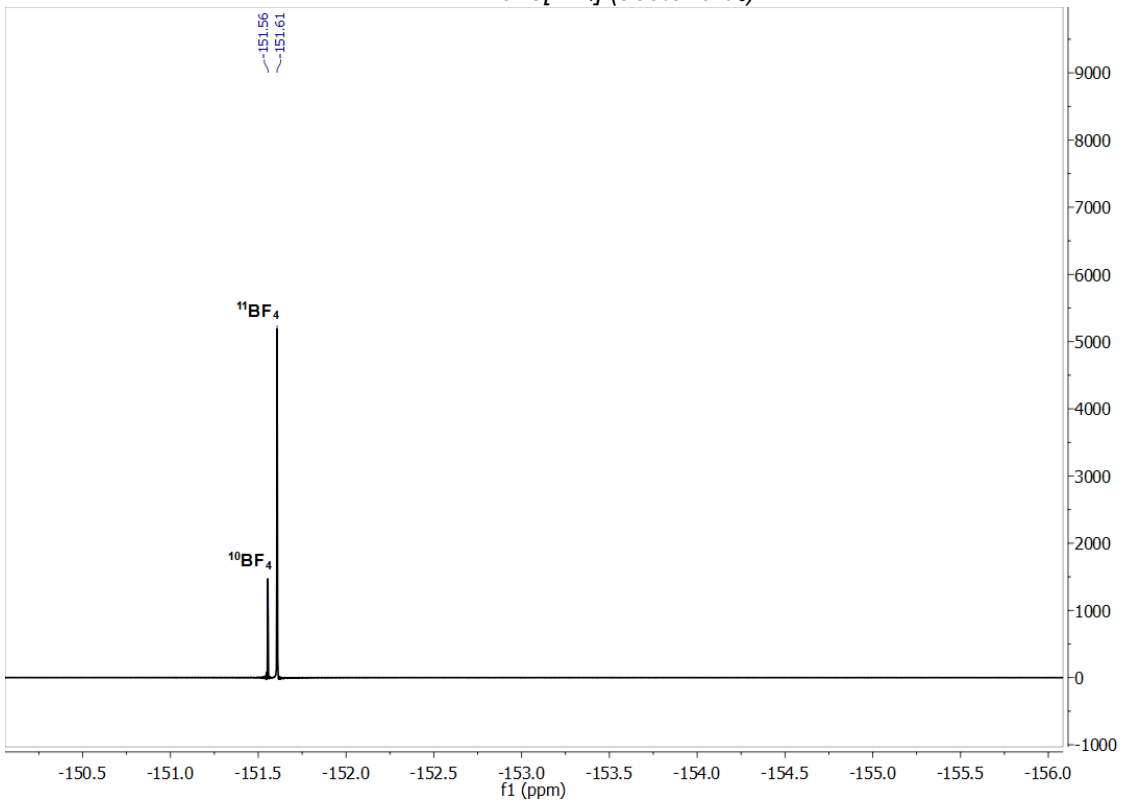
*HSQC of **3**[BF_4] and **3**[PF_6] (acetone-d_6)*



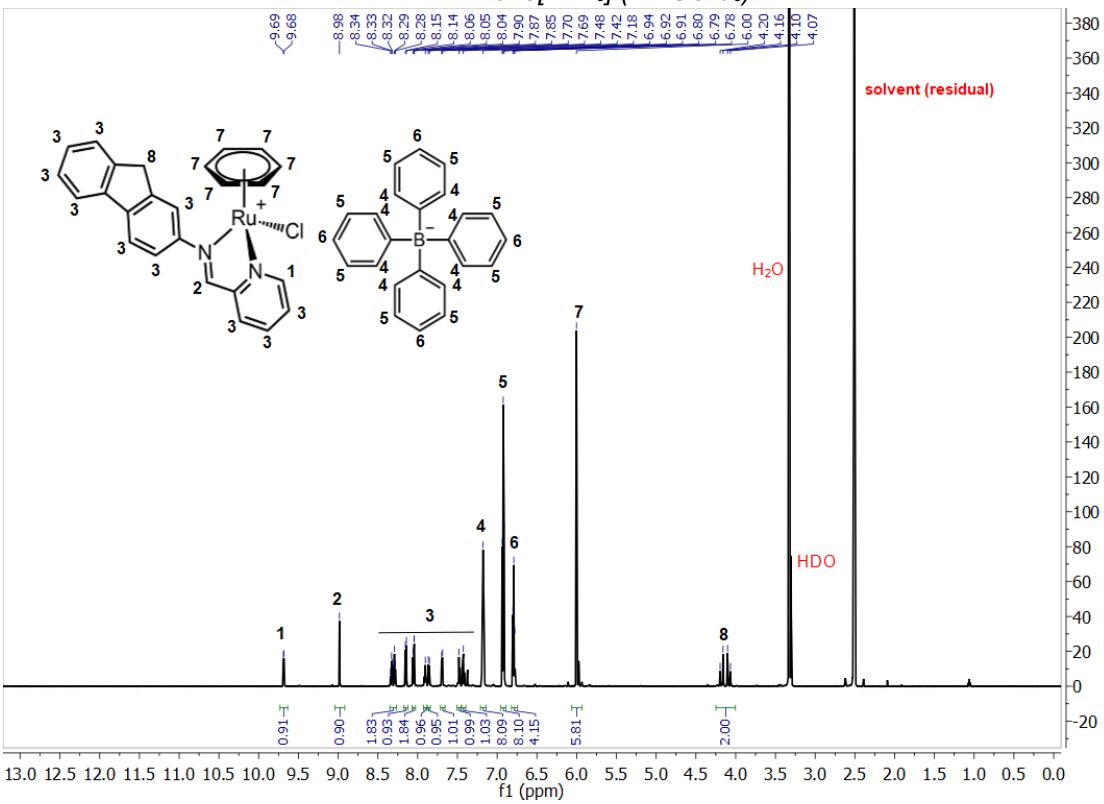
*^{19}F NMR of **3**[PF_6] (acetone-d_6)*



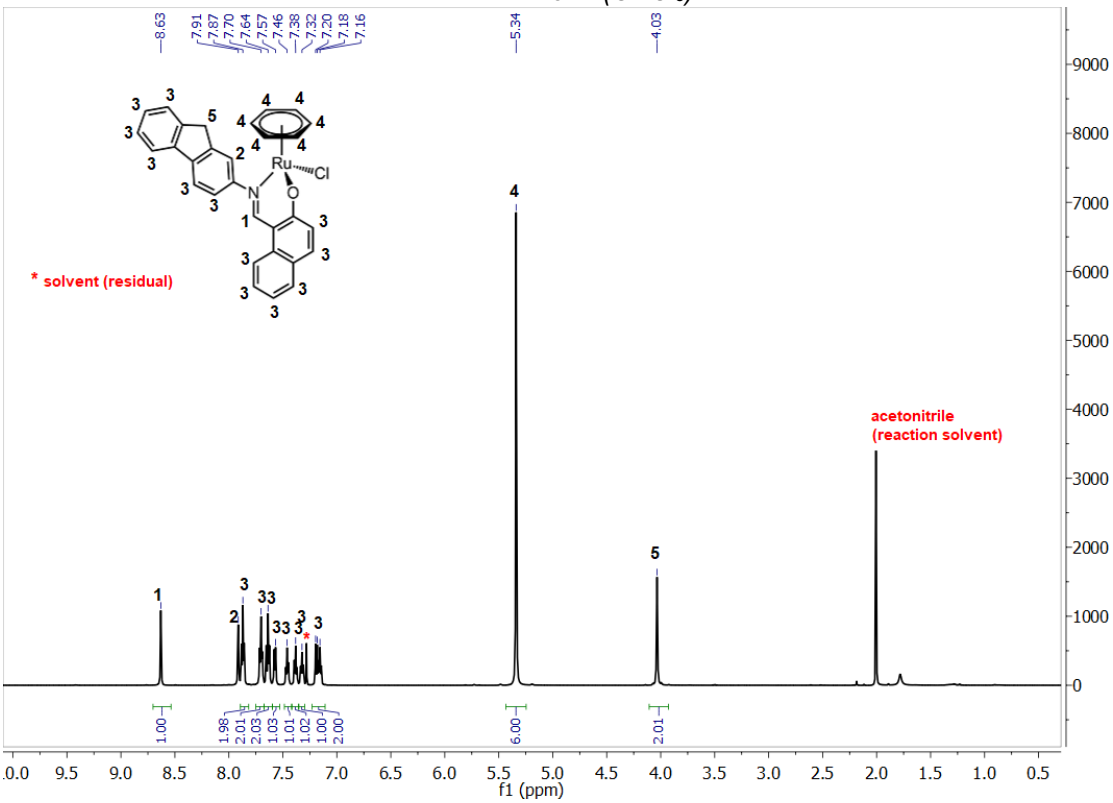
*^{19}F NMR of **3**[BF_4] (acetone-d_6)*



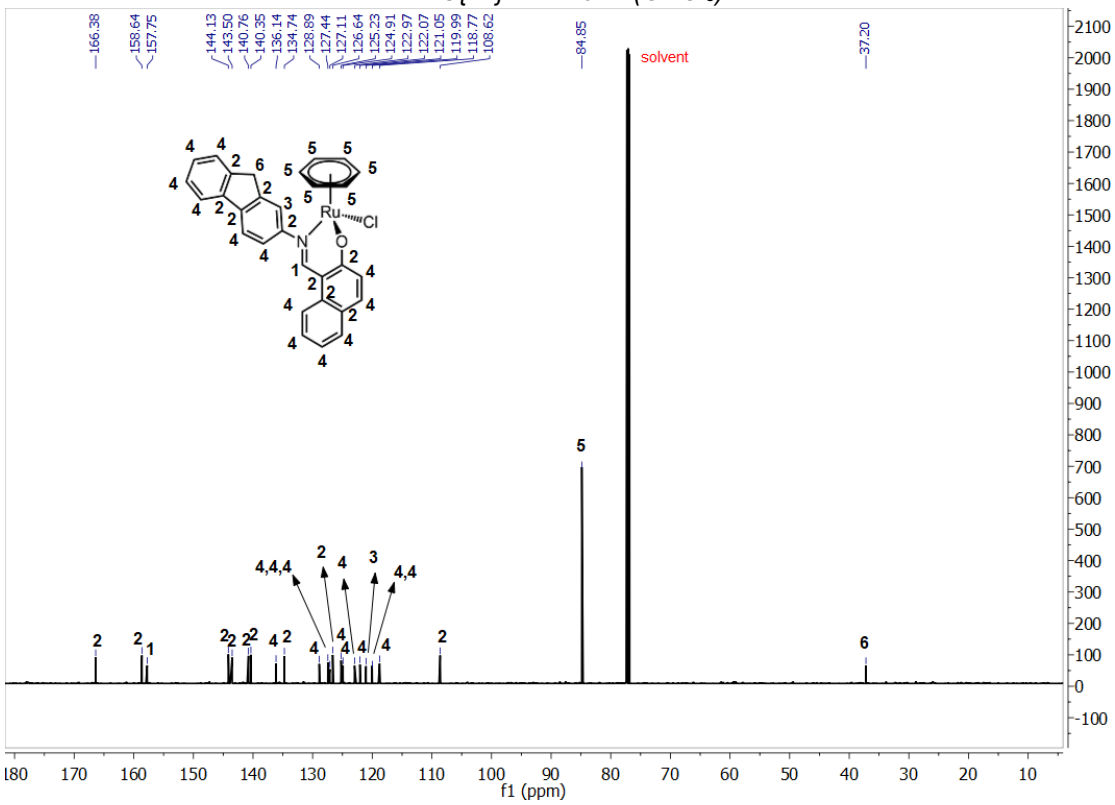
*^{1}H NMR of **3**[BPh$_6$] (DMSO-d$_6$)*



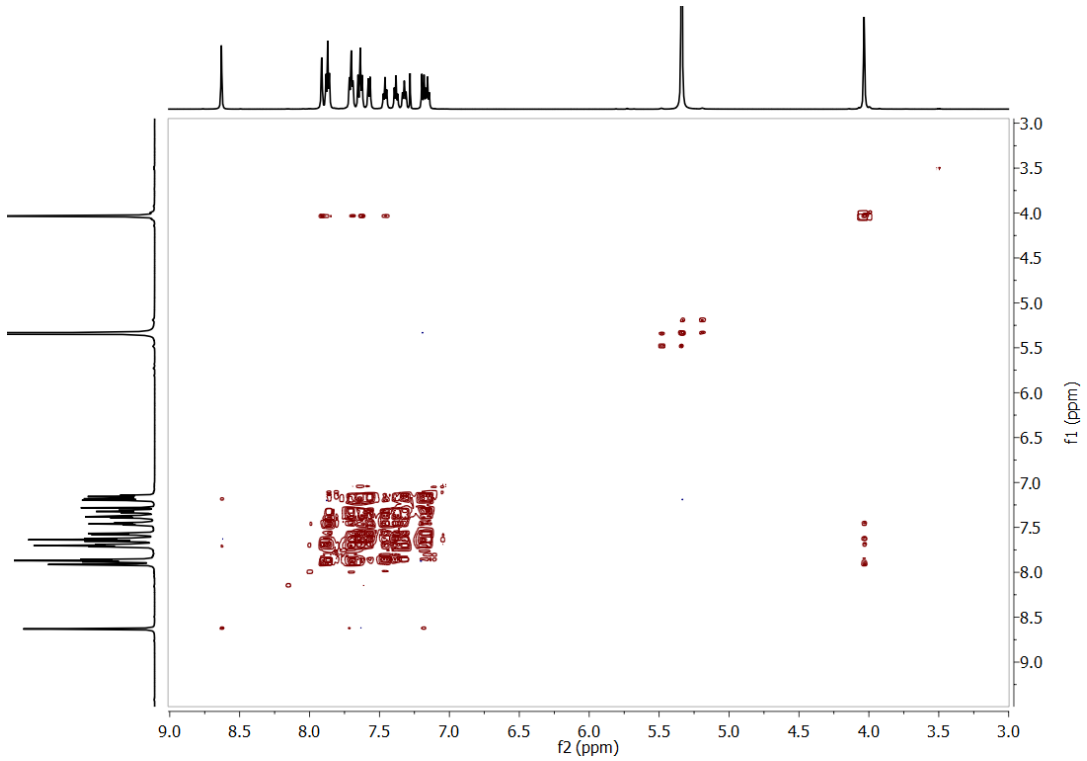
*^{1}H NMR of **4** (CDCl$_3$)*



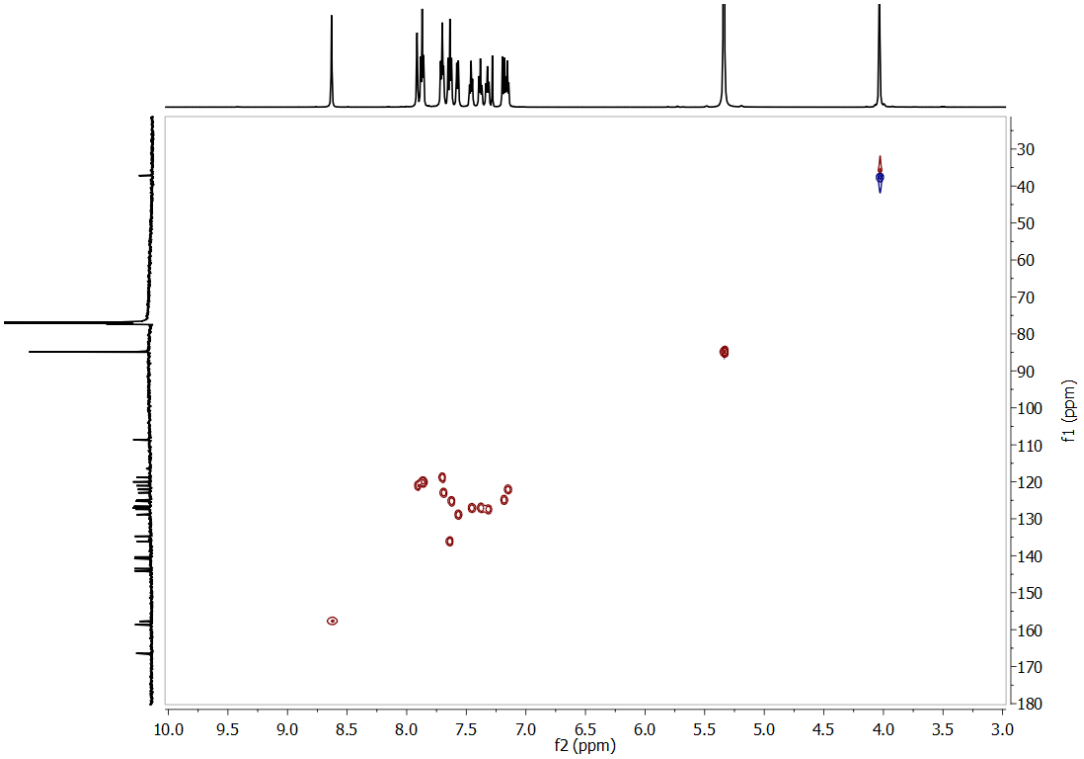
*$^{13}C\{^{1}H\}$ NMR of **4** ($CDCl_3$)*



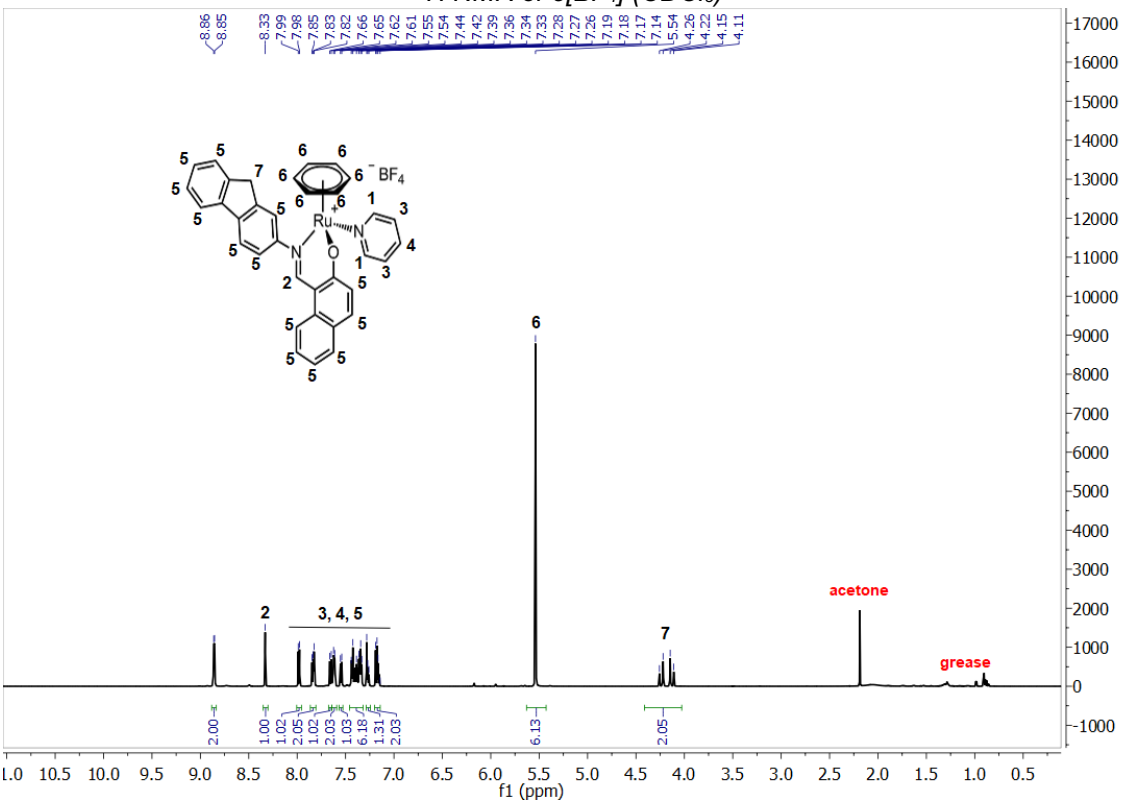
*COSY of **4** ($CDCl_3$)*



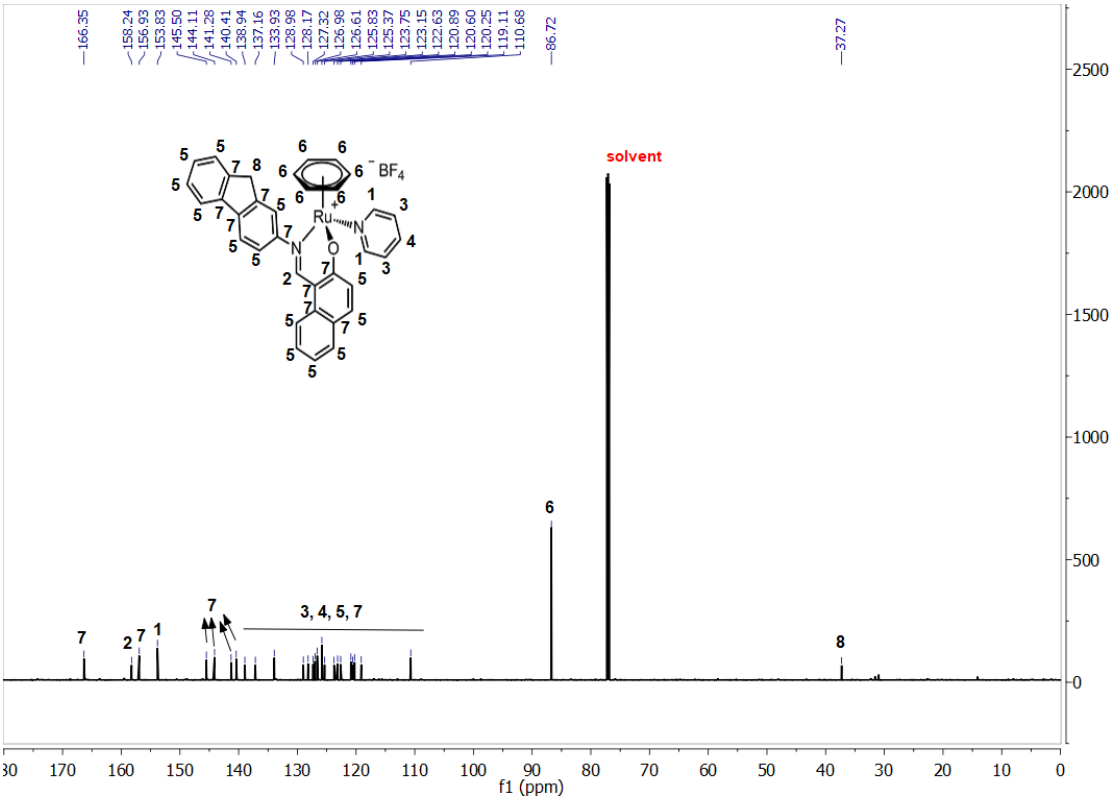
*HSQC of **4** ($CDCl_3$)*



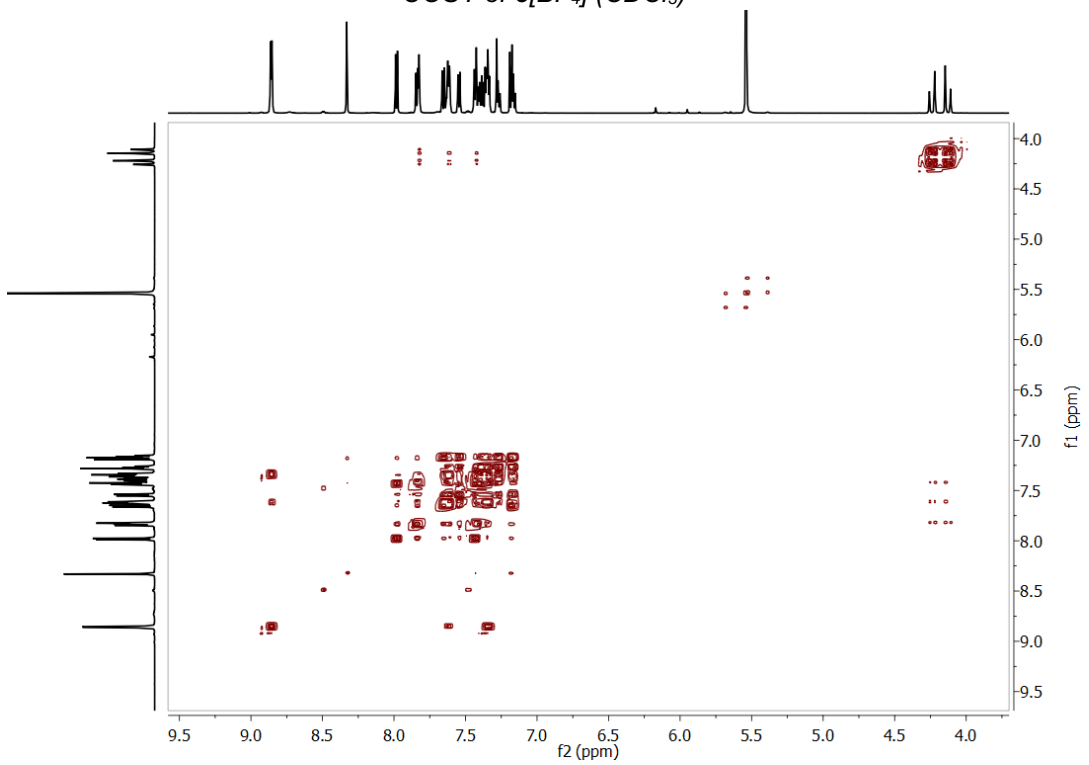
*^{1}H NMR of **5**[BF_4] ($CDCl_3$)*



*$^{13}C\{^{1}H\}$ NMR of **5**[BF_4] ($CDCl_3$)*



*COSY of **5**[BF_4] ($CDCl_3$)*



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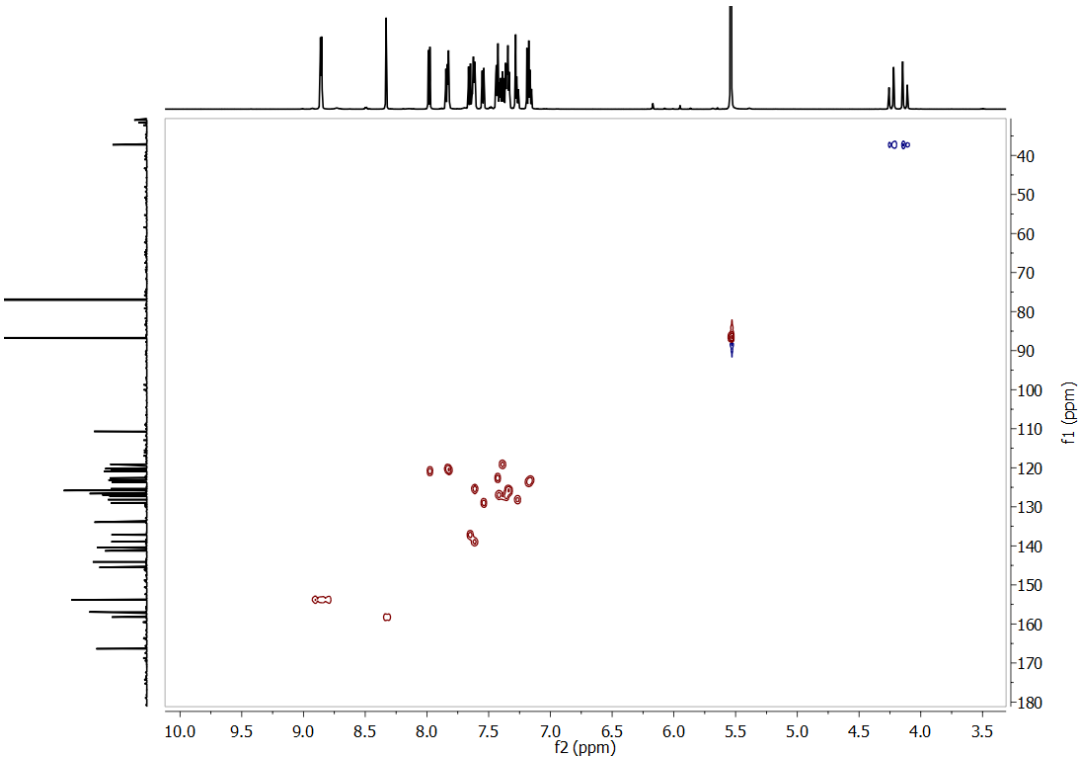

*^{19}F NMR of **5**[BF_4] ($CDCl_3$)*